case for thymidylate kinase and other nucleoside and nucleotide kinases, this target is inside the cell. It is well known that such substances (nucleoside polyphosphates) are first degraded to their nucleosides and can then enter the cell. In the case of TP_5A, this would mean that thymidine and adenosine would enter cells of persons treated with TP_5A in any conceivable form. These substances are known to have no useful intracellular therapeutic activity.

Thus, claim 31 provides a novel and inventive method to obtain inhibitors which may be used according to claim 35 for the inhibition of virus replication or for treating cancer. Therefore, the use of said inhibitors according to claim 35 is inventive.

3. REQUESTS

With the above explanations to the claims we feel that the applicant has met the requirements of the PCT. It is therefore requested that Examiner's objections be withdrawn and that a favorable IPER be issued.

Dr. Joachim Wachenfeld
European Patent Attorney

Enc.:
Proposed new set of claims, in triplicate

New set of claims

1. A method for the production of a polypeptide having or having enhanced kinase activity for a nucleoside or nucleotide analog, said method comprising substituting, adding or deleting at least one amino acid of a protein having nucleoside or nucleotide kinase activity at a position in the protein where:
   (a) the amino acid is at position X_2 and/or X_3 in the consensus sequence $GX_1X_2X_3X_4GK$ of the P-loop;
   (b) the amino acid is in the LID region; and/or
   (c) the amino acid is at position 105 in the amino acid sequence of human thymidylate kinase or at a corresponding position in a protein having nucleoside or nucleotide kinase activity.

2. The method of claim 1, wherein said nucleoside is adenosine, cytidine, guanosine, thymidine or uridine or based on any of these.

3. The method of claim 1 or 2, wherein said nucleotide is a nucleoside monophosphate.

4. The method of claim 3, wherein said nucleoside monophosphate is thymidylate.

5. The method of any one of claims 1 to 4, wherein said protein is derived from a eukaryotic or prokaryotic organism.

6. The method of claim 5, wherein said organism is human or a yeast.

7. The method of any one of claims 1 to 6, wherein said protein comprises the amino acid sequence of any one of SEQ ID NOS: 1 to 13 or a fragment thereof.

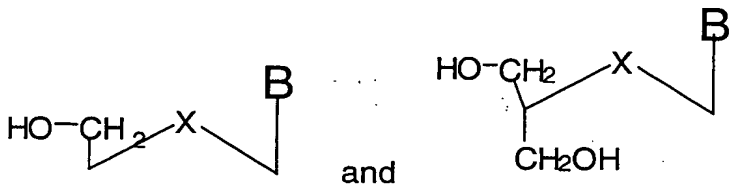
8. The method of any one of claims 1 to 7, wherein said amino acid which is substituted or added in (a) is glutamic acid, glycine or lysine and the amino acid substituted in (c) is tyrosine.

9. The method of any one of claims 1 to 8, wherein said amino acid which is substituted or added in (b) is a basic amino acid, preferably arginine.

10. The method of any one of claims 1 to 9 where the LID has the consensus sequence R/KXXXXXERYEXXXXQ.

11. The method of any one of claims 1 to 10, wherein said polypeptide exhibits kinase activity for a nucleoside or nucleotide analog which is higher than that of the/a corresponding wild type eukaryotic, preferably human enzyme.

12. The method of any one of claims 1 to 11, wherein said nucleoside analog is AZT, d4T or has the following structure:



wherein B is any nucleobase or analog thereof, and X is O, CH_2, NH or S.

13. The method of any one of claims 1 to 12, wherein the amino acid substitution(s) result in the P-loop and/or in the LID region of a bacterial nucleoside or nucleotide kinase, preferably those of the TmpK of E.coli.

14. A polynucleotide encoding the polypeptide obtainable by the method of any one of claims 1 to 13.

15. A vector containing the polynucleotide of claim 14.

16. The vector of claim 15, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

17. The vector of claim 15 or 16, which is a gene transfer or a gene targeting vector.

18. A host cell genetically engineered with the vector of any one of claims 15 to 17.

19. A method for producing a polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog comprising
    (a) culturing the host cell of claim 18, and
    (b) recovering said polypeptide from the culture.

20. A method for producing cells capable of expressing a polypeptide having nucleoside or nucleotide kinase activity for nucleoside or nucleotide analogs comprising genetically engineering cells with the polynucleotide of claim 14, or with the vector of any one of claims 15 to 17.

21. A polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog encoded by polynucleotide of claim 14, obtainable by the method of any one of claims 1 to 13 or 19 or from cells produced by the method of claim 20 or comprising a biologically active fragment of any of these.

22. An antibody specifically recognizing the polypeptide of claim 21.

23. A composition comprising
    (a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide; or

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17;

(c) optionally a nucleoside or nucleotide analog; and

(d) optionally a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein said protein is a bacterial nucleoside or nucleotide kinase.

25. The composition of claim 23 or 24, wherein said protein is a bacterial TmpK.

26. The composition of any one of claims 23 to 25, wherein said protein has at least the P-loop and/or the LID region of E.coli TmpK.

27. The composition of claim 25 or 26, wherein said TmpK comprises the amino acid sequence shown in SEQ ID NO: 4 or a biologically active fragment thereof.

28. A kit comprising the polynucleotide of claim 14, the vector of any one of claims 15 to 17, the protein of claim 21 or the antibody of claim 22, and optionally a nucleoside or nucleotide analog.

29. A method for identifying an inhibitor of a nucleoside or nucleotide kinase comprising the steps of:

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase.

30. A method for identifying a nucleoside or nucleotide based prodrug comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug.

31. A method for the production of a pharmaceutical composition comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase, or

(a') contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b') detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug; and

(c) formulating the inhibitor identified in step (b) or the nucleoside or nucleotide analog identified in step (b') in a pharmaceutically acceptable form.

32. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17; and/or

(c) the nucleoside or nucleotide analog identified in the method of claim 30

for the preparation of a pharmaceutical composition for the activation of nucleoside or nucleotide analogs or nucleoside or nucleotide based prodrugs and/or for the treatment of viral infections and/or diseases or cancer.

33. The use of claim 32, wherein said activation results in a cytotoxic nucleoside or nucleotide.

34. The use of claim 32 or 33, wherein said viral infection is HIV infection.

35. Use of the inhibitor obtainable by the method of claim 31 for the preparation of a pharmaceutical composition for inhibiting virus replication or for treating cancer.

36. A method for the preparative synthesis of a nucleoside phosphate analog comprising:

(a) using a polynucleotide of claim 14 or as defined in any one of claim 23 to 27 in a noncellular system or in a cell ex vivo, and

(b) formulating the cells modified in step (a) in a pharmaceutically acceptable form.

37. The composition of any one of claims 23 to 27 or the use of any one of claims 32 to 34, wherein said composition is a pharmaceutical composition and further comprises or is designed to be administered with a nucleoside or nucleotide analog, preferably AZT or d4T.

38. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17

for the preparation of nucleoside phosphates or analogs and derivatives thereof.

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New set of claims

1. A method for the production of a polypeptide having or having enhanced kinase activity for a nucleoside or nucleotide analog, said method comprising substituting, adding or deleting at least one amino acid of a protein having nucleoside or nucleotide kinase activity at a position in the protein where:
   (a) the amino acid is at position X_2 and/or X_3 in the consensus sequence $GX_1X_2X_3X_4GK$ of the P-loop;
   (b) the amino acid is in the LID region; and/or
   (c) the amino acid is at position 105 in the amino acid sequence of human thymidylate kinase or at a corresponding position in a protein having nucleoside or nucleotide kinase activity.

2. The method of claim 1, wherein said nucleoside is adenosine, cytidine, guanosine, thymidine or uridine or based on any of these.

3. The method of claim 1 or 2, wherein said nucleotide is a nucleoside monophosphate.

4. The method of claim 3, wherein said nucleoside monophosphate is thymidylate.

5. The method of any one of claims 1 to 4, wherein said protein is derived from a eukaryotic or prokaryotic organism.

6. The method of claim 5, wherein said organism is human or a yeast.

7. The method of any one of claims 1 to 6, wherein said protein comprises the amino acid sequence of any one of SEQ ID NOS: 1 to 13 or a fragment thereof.

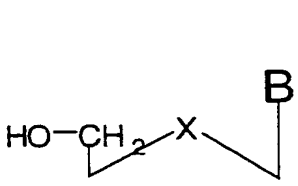
8. The method of any one of claims 1 to 7, wherein said amino acid which is substituted or added in (a) is glutamic acid, glycine or lysine and the amino acid substituted in (c) is tyrosine.

9. The method of any one of claims 1 to 8, wherein said amino acid which is substituted or added in (b) is a basic amino acid, preferably arginine.

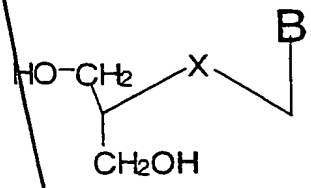
10. The method of any one of claims 1 to 9 where the LID has the consensus sequence R/KXXXXXERYEXXXXQ.

11. The method of any one of claims 1 to 10, wherein said polypeptide exhibits kinase activity for a nucleoside or nucleotide analog which is higher than that of the/a corresponding wild type eukaryotic, preferably human enzyme.

12. The method of any one of claims 1 to 11, wherein said nucleoside analog is AZT, d4T or has the following structure:



and



wherein B is any nucleobase or analog thereof, and X is O, CH_2, NH or S.

13. The method of any one of claims 1 to 12, wherein the amino acid substitution(s) result in the P-loop and/or in the LID region of a bacterial nucleoside or nucleotide kinase, preferably those of the TmpK of E.coli.

14. A polynucleotide encoding the polypeptide obtainable by the method of any one of claims 1 to 13.

15. A vector containing the polynucleotide of claim 14.

16. The vector of claim 15, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

17. The vector of claim 15 or 16, which is a gene transfer or a gene targeting vector.

18. A host cell genetically engineered with the vector of any one of claims 15 to 17.

19. A method for producing a polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog comprising
(a) culturing the host cell of claim 18, and
(b) recovering said polypeptide from the culture.

20. A method for producing cells capable of expressing a polypeptide having nucleoside or nucleotide kinase activity for nucleoside or nucleotide analogs comprising genetically engineering cells with the polynucleotide of claim 14, or with the vector of any one of claims 15 to 17.

21. A polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog encoded by polynucleotide of claim 14, obtainable by the method of any one of claims 1 to 13 or 19 or from cells produced by the method of claim 20 or comprising a biologically active fragment of any of these.

22. An antibody specifically recognizing the polypeptide of claim 21.

23. A composition comprising
(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide; or

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17;

(c) optionally a nucleoside or nucleotide analog; and

(d) optionally a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein said protein is a bacterial nucleoside or nucleotide kinase.

25. The composition of claim 23 or 24, wherein said protein is a bacterial TmpK.

26. The composition of any one of claims 23 to 25, wherein said protein has at least the P-loop and/or the LID region of E.coli TmpK.

27. The composition of claim 25 or 26, wherein said TmpK comprises the amino acid sequence shown in SEQ ID NO: 4 or a biologically active fragment thereof.

28. A kit comprising the polynucleotide of claim 14, the vector of any one of claims 15 to 17, the protein of claim 21 or the antibody of claim 22, and optionally a nucleoside or nucleotide analog.

29. A method for identifying an inhibitor of a nucleoside or nucleotide kinase comprising the steps of:

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase.

30. A method for identifying a nucleoside or nucleotide based prodrug comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug.

31. A method for the production of a pharmaceutical composition comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase, or

(a') contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b') detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug; and

(c) formulating the inhibitor identified in step (b) or the nucleoside or nucleotide analog identified in step (b') in a pharmaceutically acceptable form.

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32. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17; and/or

(c) the nucleoside or nucleotide analog identified in the method of claim 30

for the preparation of a pharmaceutical composition for the activation of nucleoside or nucleotide analogs or nucleoside or nucleotide based prodrugs and/or for the treatment of viral infections and/or diseases or cancer.

33. The use of claim 32, wherein said activation results in a cytotoxic nucleoside or nucleotide.

34. The use of claim 32 or 33, wherein said viral infection is HIV infection.

35. Use of the inhibitor obtainable by the method of claim 31 for the preparation of a pharmaceutical composition for inhibiting virus replication or for treating cancer.

36. A method for the preparative synthesis of a nucleoside phosphate analog comprising:

(a) using a polynucleotide of claim 14 or as defined in any one of claim 23 to 27 in a noncellular system or in a cell ex vivo, and

(b) formulating the cells modified in step (a) in a pharmaceutically acceptable form.

37. The composition of any one of claims 23 to 27 or the use of any one of claims 32 to 34, wherein said composition is a pharmaceutical composition and further comprises or is designed to be administered with a nucleoside or nucleotide analog, preferably AZT or d4T.

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38. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17

for the preparation of nucleoside phosphates or analogs and derivatives thereof.

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